2004 Annual Report

An Evaluation of the Reproductive Success of Natural-Origin, Hatchery-Origin, and Kelt Steelhead in the Columbia Basin
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ABSTRACT

Iteroparity, the ability to repeat spawn, is a life history strategy that is expressed by some species from the family Salmonidae. Rates of repeat spawning for post-development Columbia River steelhead *Oncorhynchus mykiss* populations range from 1.6 to 17%. It is expected that currently observed iteroparity rates for wild steelhead in the Basin are severely depressed due to development and operation of the hydropower system and various additional anthropogenic factors. With the recent Endangered Species Act listing of all Columbia River steelhead populations, there is a pressing need for conservation measures to enhance the natural population abundance. Increasing the expression of repeat spawning rates using fish culturing methods could be a viable technique to assist the recovery of depressed steelhead populations, and could help reestablish this naturally occurring life history trait. Reconditioning is the process of culturing post-spawned steelhead (kelts) in a captive environment until they are able to reinitiate feeding, growth, and redevelop mature gonads.

Prior to an implementation of a large-scale steelhead kelt reconditioning program, it is important to evaluate the reproductive success of reconditioned kelts relative to their hatchery and natural counterparts. A potential concern is that reconditioned steelhead kelts may not be as reproductively successful as first time spawners. To assess this issue, three new sites (Satus Creek,WA; Omak Creek, WA: and Shitike Creek, OR) have been chosen to replicate studies of relative reproductive success of each variant of steelhead. Technological advances in DNA-typing make direct measurement of reproductive success using pedigree analysis practical. Employing these new techniques, our study will directly measure the reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead in natural stream settings. This will yield quantitative data replicated geographically and temporally that will add resolution to the issue.

In 2004 we succeeded in coordinating and determining the best reconditioning sites based on our resources as well as purchasing equipment and supplies to long-term recondition steelhead kelts at the three additional sites. So far, our efforts at coordination and approval from various agencies involved has gone well and appears to be on track towards a successful 2005 field season. Development of an operations plan to facilitate successful coordination at the three reconditioning facilities was also produced.

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1.0 INTRODUCTION

1.1 Background

Iteroparity, the ability to repeat spawn, is a natural life history strategy that is expressed by *Oncorhynchus mykiss,* with rates estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia 1994-96 (Savvaitova et al. 1996). Historical rates for the Columbia River have not been accurately documented but outmigrating steelhead averaged 58% of the total upstream runs in the Clackamas River from 1956 to 1964 (Gunsolus and Eicher 1970). Current iteroparity rates for Columbia River basin steelhead are considerably lower, due largely to high mortality of downstream migrating kelts (post-spawn steelhead) at hydropower dams (Evans and Beaty 2000, 2001; Evans 2002; Wertheimer et al. 2002), and potentially inherent differences in iteroparity rate based on latitudinal and inland distance effects (Withler 1966; Bell 1980; Fleming 1998). The highest recent estimates of repeat spawners from the Columbia River Basin were in the Kalama River (tributary of the unimpounded lower Columbia River) have exceeded 17% (NMFS 1996). Farther upstream, 4.6% of the summer run in the Hood River (above only one mainstem dam) are repeat spawners (J. Newton, ODFW, pers. comm.). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1984). Summer steelhead in the South Fork Walla Walla River have expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.), whereas repeat spawners composed only 1.6% of the Yakima River wild run (from data in Hockersmith et al. 1995) and 1.5% of the Columbia River run upstream from Priest Rapids Dam (L. Brown, WDFW, unpubl. data).

The recent Endangered Species Act (ESA) listing of all Columbia River Basin steelhead populations has prompted interest in developing artificial kelt reconditioning methods for wild steelhead populations that will take advantage of this natural life history trait to help bolster diminishing natural populations. Reviews on this subject (Hatch et al. 2002 and 2003) provide strong support of the benefits of kelt reconditioning to address population demographic and genetic issues in steelhead recovery. To evaluate the feasibility of kelt reconditioning the Yakima/Klickitat Fisheries Project (YKFP) in collaboration with the Columbia River Inter-Tribal Fish Commission (CRITFC) have been capturing wild emigrating kelt steelhead from the Yakima River and experimenting with several artificial kelt reconditioning methods since 1999 at Prosser Hatchery (BPA Project 200001700).

Kelts reconditioned during this project have substantially bolstered the number of repeat spawners in study streams. Valuable knowledge regarding kelt husbandry, food type preferences, condition, and rearing environments have been obtained during the research endeavor. Since the project's inception, 20-30% of the total annual steelhead migration has been successfully reconditioned, and radio telemetry studies have demonstrated successful spawning migrations and redd construction. In terms of numbers, an additional 100-200 reconditioned steelhead females could available to spawn a second time (a projected 300,000-600,000 additional eggs at an estimated 3,000 eggs per female) each year in the Yakima River. However, the contribution of these repeat spawners to the total population recruitment has yet to be determined.

1.2 Rationale

In the Columbia River Basin over 100 anadromous fish hatcheries release approximately 185 million juveniles per year that accounts for 75% of the anadromous fish production in the basin (NPPC 1999). The hatchery system grew from mitigation for hydro-system development and harvest augmentation to conservation programs with goals of rebuilding depleted populations. The changing goals of hatchery programs has created a controversy surrounding the conservation utility of surplus hatchery origin fish, the reproductive contribution of hatchery-origin fish in the wild, and the use of supplementation as a restoration tool (ISAB 2002). The ISAB (2002) framed this debate with three questions: 1.) Do the hatchery-origin salmon spawn; 2.) Do the hatchery-origin salmon produce offspring equally as well as wild salmon; and, 3.) Do interbreeding between hatchery-origin and wild salmon affect the fitness of the wild population? The steelhead's ability to repeat spawn is unique among the Columbia River salmonids and may offer an alternative strategy to bolster natural production through the promotion of this natural life history trait. Differing from more conventional hatchery production, post spawn steelhead adults can be collected, reconditioned and then released to spawn again. Granted this approach is drastically different from traditional hatchery strategies but many of the same concerns still exist.

Specific questions regarding the success of artificially reconditioning kelt steelhead include: do reconditioned kelts produce viable offspring that contribute to recruitment, how does kelt reproductive success compare with natural first time spawners, and how does kelt reproductive success compare with hatchery origin hatchery spawners? Answers to these questions will be important in the determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin. We have established that kelt reconditioning is possible and have demonstrated successful spawning migrations and redd construction (BPA Project 200001700). However, the reproductive success of reconditioned kelts needs to be explored to assess the net benefit of this program. In addition, comparisons to natural origin first time spawners can be used to evaluate reconditioned kelt contributions relative to a first time spawner baseline, and comparisons with hatchery origin steelhead is important to evaluate if kelt reconditioning is a viable alternative to more traditional hatchery supplementation.

Through observational studies hatchery-origin females generally showed greater reproductive abilities than hatchery-origin males and in most cases there are few differences in reproductive abilities and performance between females of hatchery and natural-origin (Fleming and Gross 1993, Fleming et al 1997, Fleming and Petersson 2001). This led Fleming and Gross (1993) to conclude that introducing hatchery-origin females rather than males may be an important technique for rebuilding wild populations when using hatchery fish. This could have important implications with kelt reconditioning since greater than 85% of the post spawn collected individuals are female (Evans 2002; Hatch et al. 2002). Naturally occurring female iteroparity is essentially analogous to cryopreservation of males in other salmon conservation programs in the Columbia River Basin. In addition, the fact that females are naturally able to reproduce with males during different years increases the probability of gene flow between and among cohorts or year classes. This has a direct theoretical benefit in the form of increasing the number of breeders (N_b), and the effective population size (N_e) during each spawning season, thus contributing to increased population viability and persistence, crucial to threatened and endangered fish restoration. Rather than a genetic hazard, experimental reconditioning should be viewed as a potential demographic and population genetic enhancement measure, aimed at restoring a recently jeopardized, but naturally occurring evolutionarily stable life history strategy.

Technological advances in DNA-typing make direct measurement of reproductive success using pedigree analysis practical. Employing these new techniques, our proposed study will directly measure the reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead in natural stream settings. This will yield quantitative data replicated geographically and temporally that will add resolution to the issue.

2.0 Objectives and Tasks

2.1 Area and Facilities

The Columbia River Inter-Tribal Fish Commission from their office in Portland, Oregon will coordinate this project. Field collections will be made on Shitike Creek, a tributary to the Deschutes River; Omak Creek, a tributary to the Okanagon River; and Satus Creek a tributary to the Yakima River. Reconditioning will be conducted at Warm Springs National Fish Hatchery

(Shitike fish), Cassimer Bar Fish Hatchery (Omak fish), and Prosser Fish Hatchery (Satus fish). Genetic analysis will be performed at the Collaborative Center for Applied Fish Science in Hagerman, Idaho.

2.2 Objectives

In order to evaluate the feasibility of kelt reconditioning as a potential recovery and restoration strategy for wild steelhead in the Columbia River basin, this project was designed to satisfy the following research objectives:

1. **Plan and coordinate all aspects of project implementation including permitting, subcontracting, and logistics.**

2. **Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at Shitike Creek, Omak Creek, and a tributary of the Yakima River using pedigree analysis.**

3. **Apply kelt steelhead reconditioning techniques at selected streams to post-spawners for release back into study streams.**

2.3 Planning and Coordination

1. **Plan and coordinate all aspects of project implementation including permitting, subcontracting, and logistics.**

2.3.1 Steelhead Prespawner Collection

A critical component of this project includes sampling all returning adult steelhead. Weir locations and placements must be determined for each site. Necessary permits including county, state, federal, and tribal permits must be obtained. Testing of weirs for collection efficiency and operation will be finished by late 2004. Weir material and fabrication will have to be done at the Yakima River Basin site. Weirs are already available at Shitike and Omak creeks however; some additional replacement equipment may need to be purchased.

A suitable stream in the Yakima River Basin will be chosen and a weir site selected. The stream selection criteria will include a relatively low abundance of adult steelhead in the stream (~200). The stream must have hydrological conditions that are favorable to using a weir during the steelhead migration. The stream must also have a suitable location for a rotary screw trap or some other juvenile trap.

2.3.2 Steelhead Kelt Collection

Two approaches were investigated for reconditioning. The first approach was a centralized reconditioning facility where individuals from each study stream would be transported to a single facility for reconditioning and then transported back to the study stream for release. Centralizing this portion of the project could reduce risk and maximize the probability of reconditioning due to the Prosser Hatchery's experience. Challenges to this approach include: screening fish for pathogens and coordinating and permitting with appropriate agencies, and transporting kelt steelhead from collection weirs to the Hatchery. As a result of pathogen concerns this approach has been abandoned in lieu of the second approach, reconditioning within each subbasin.

The immediate advantage of reconditioning within each subbasin is that it reduces the disease transfer concern associated with trucking adult fish out of subbasins. Challenges associated with this approach include locating suitable facilities for reconditioning, additional staff training, and additional sites to install equipment at. Kelt steelhead from Omak Creek will be reconditioned at Cassimer Bar, a facility that is now rearing steelhead for a Omak Creek Localized Broodstock Project. Yakima Basin fish will be reconditioned at Prosser Hatchery and Shitike Creek fish will be reconditioned at the Warm Springs National Hatchery.

2.3.3 Steelhead Kelt Reconditioning

Additionally, kelt steelhead will be collected from each stream. Genetic analysis will be performed at the Collaborative Center for Applied Fish Science in Hagerman, Idaho. After collecting steelhead kelts they will then be transported to the following sites for reconditioning: Warm Spring National Fish Hatchery (Shitike Creek steelhead kelts), Cassimer Bar Hatchery (Omak Creek steelhead kelts), and Prosser Hatchery (Satus Creek steelhead kelts). Before release to reconditioning tanks we will collect data on weight, length, and condition as well as administration of Ivermectin, PIT-tags, and formalin for parasitic fungal treatment then promptly begin feeding kelts krill (starter feed). Steelhead kelts will be reconditioned for a 5-9 month time span before release to insure rematuration. Based on weight change during captivity, we will classify surviving specimens as either feeders or non-feeders. Fish in the experiments will be released to coincide with natural spawn timing. Growth measurement data and rematuration status will also recorded on all released individuals. Overall success of the reconditioning process will be based on the proportion of fish that survived the reconditioning process and the number of fish that successfully rematured (based on ultrasound examinations).

2.3.4 Juvenile *O. mykiss* Collection

The resulting steelhead progeny will be collected from each stream using a floating rotary traps (screw traps) and each outmigrating juvenile will be sampled for DNA profiling. Trap locations and placements must be obtained for each site. Necessary permits including county, state, federal, and tribal permits must be obtained. Testing of traps for collection efficiency and operation will be done. A trap for the Yakima River site will have to be procured. Traps are already available at Shitike and Omak creeks.

2.3.5 Resident *O. mykiss* Population Collection

Resident trout can contribute to the anadromous cohort from a stream and therefore it is important to account for juveniles with undetermined linage by surveying resident populations. Collections of resident trout will be made using the rotary screw trap. Tissue samples will be collected, but genotyping will not be finished until next year.

2.3.6 Genotyping

Test runs will be done in late 2004 to allow us to optimize loci and multiplex sets. This work will increase throughput during years when high volumes of samples will be analyzed.

2.3.7 Project Coordination

Geographic replication of specimen collection, artificial reconditioning of post-spawn steelhead, and state-of-the-art genetic analysis are all procedures that will need coordination. Three different tribal fishery staffs will be conducting field collections and CRITFC will coordinate activities. The target species is listed under the Endangered Species Act (ESA) in all areas above Bonneville Dam, which invokes a federal permitting process. Due to the high level of project complexity and limited available project funds from BPA, this project concentrated on planning and coordination in FY04. Major coordination tasks include:

- -Formal project approval from Tribal Fish and Wildlife Committees;
- -Subcontracting with individual tribes including, The Yakama Nation, The Confederated Tribes of Warm Springs and the Confederated Tribes of the Colville Reservation.
- -Procurement of weir materials, rotary screw trap(s), holding tanks, reconditioning tanks, PIT tag detector systems, two ultra-sound machines, and miscellaneous additional field gear.
- -Equipment testing.
- -Development of protocols for sampling, collecting, tagging, handling
- -Permitting, including all necessary Federal, Tribal, State, County, etc.
- -Development of annual reports and statements of work.

2.4 Reproductive Success

Objective 2. Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at Shitike Creek, Omak Creek, and a tributary of the Yakima River using pedigree analysis.

2.4.1 Parentage Assignment

Highly polymorphic microsatellite loci have become the marker of choice for parentage and population studies due to the potential for differentiating closely related populations and accurate parentage assignment (Bernatchez and

Duchesne 2000; Eldridge et al. 2002; Estoup et al. 1998; Letcher and King 2001; Norris et al. 1999; O'Reilly et al 1998). Utilizing microsatellite loci optimized for steelhead studies (Narum et al. in review), we plan to determine the reproductive success of wild, hatchery, and kelt steelhead from three replicate sites in the Columbia Basin. The process will be comprised of four steps: 1) collect year 2005 samples from each of three study sites (all adult returns over three selected weirs, smolt progeny from screwtraps, and adult resident rainbow trout), 2) generate microsatellite genotypes from all samples taken in 2005 and perform parentage assignments, 3) collect adult returns of brood year 2005 steelhead (annually in 2007, 2008, and 2009), and 4) generate microsatellite genotypes of annual adult returns and assign parentage. Specifically, we will attempt to assign the parentage of juvenile progeny (and subsequent adults) to adult collections of wild, hatchery, or kelt steelhead. This method will allow us to quantify not only the reproductive contribution of individual fish, but also quantify the adult returns related to each parental category of steelhead.

2.4.2 Generalized Sampling Scheme

The sampling scheme that has been devised includes one pretreatment / control and 2 temporal replicates per site and thus require a 12-year study period. This aspect of the study clarifies a concern from the ISRP regarding temporal and spatial replication in our study and also illustrates a general concern expressed by the ISRP that studies designed to address the RFS require a long-term commitment. We have estimated the number of samples that would be genotyped to follow three year-classes (1 pretreatment and 2 treatment) through their life span. Using age structure data we have allocated samples across collection years to minimize cost while maintaining temporal replication. The field collection portion of the study remains unchanged, thus we will likely collect more tissue samples in the field than will be genotyped in the laboratory.

Steelhead in the Columbia River Basin exhibit a variety of life-history strategies that leads to sampling challenges. We used data from the Umatilla River as a guide to developing our generalized sampling scheme for all of the study streams. Prior to implementation we will use stream specific estimates, if available, and adjust accordingly. Based on these data we will concentrate our sampling effort on collecting 3, 4, and 5 year-old fish with 2 to 4 year freshwater residency. We have ignored Age 1.x fish in this example because they made up a very small fraction of the run. Age 4.x fish also made up a small fraction of the run, but we anticipate that this age may comprise a larger component in our study streams. Additionally, allocating samples to the Age 4.x component does not have much effect on the total.

Below is a table that indicates which samples collected at weirs and rotary screw traps will be genotyped. This scheme provides for 2 temporal replicates and a pretreatment / control at each study stream yet minimizes the number of samples that must be genotyped. Please note that the weirs will be operated during each of the 12 years of the study and tissue samples will be collected and archived from all fish that pass. The rotary screw traps will be operated annually from 2006 through 2013 and tissue samples will be collected in a manner to represent the run. In the table below we also include estimated sample sizes by collection year and life stage per sample stream.

Table 1: Samples that will be genotyped for pedigree analysis. Age determinations will be made prior to sequencing using scale pattern analysis and/or length frequency. This scheme will be followed at each study stream, except in the Satus Creek Basin where just the BY05 and BY09 year-classes will be followed. BY=broodyear and the subscripted numerals indicate freshwater age and saltwater age separated by a period. Sample size allocations were calculated based on age structure data from the Umatilla River. The pretreatment / control year-class (*BY05*) is identified by italics.

Year	Sample Adults	Sample Juveniles	Sample Residents		# of Adults genotyped	# of juveniles genotyped	Estimated # to genotype per study stream
2005	**All Adults**		XX		300		300
2006	All Adults		XX		300		300
2006		$BY05_{2.}$				336	336
2008	$BY05_{1.2\&2.1}$	$BY05_{3.}$ $\mathrm{BY06}_{2.}$	XX		104	372	476
2009	All Adults	$BY05_{4.}$ $\mathrm{BY06}_{3.}$			300	64	364
2010	$BY05_{2.3\&3.2\&4.1}$ $\mathrm{BY06}_{2.2\&3.1}$	$\mathrm{BY06}_{4.}$			96	28	124
2011	$\mathrm{BY06}_{2.3\&3.2\&4.1}$	$\mathrm{BY09}_{2.}$			10	336	346
2012	$\mathrm{BY09}_{1.2\&2.1}$	$\mathrm{BY09}_{3.}$			104	36	140
2013	$\mathrm{BY09}_{2.2\&3.1}$	$\mathrm{BY09}_{4.}$			86	28	114
2014	$\mathrm{BY09}_{2.3\&3.2\&4.1}$				10		10
Total					1,310	1,200	2,510

2.4.3 Laboratory Techniques

Samples will be collected and stored in ethanol or lysis buffer for preservation of DNA. Samples will be shipped to the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA will be extracted from tissue samples using standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. Genomic DNA will be quantified and arrayed into 96 well plates for high throughput genotyping. The polymerase chain reaction (PCR) will be used to amplify 10-12 microsatellite loci designed from *O. mykiss*. PCR amplifications will be performed using the AmpliTaq Reagent System (Applied Biosystems®) in an MJ Research® PTC-100 thermal cycler following manufacturer's protocols. Forward PCR primers will be fluorescently labeled (Applied Biosystems®), and

PCR products genotyped using manufacture's protocols with an Applied Biosystems® model 3100 or 3730 genetic analyzer.

2.4.4 Statistical Analysis

Data will be analyzed with two specific goals: 1) to quantify gene flow between adult wild, hatchery and kelt steelhead within and between sites, using traditional population genetics tests (Hardy-Weinberg equilibrium, F statistics, assignment tests) [Genepop (Raymond and Rousset 1995); GDA (Lewis and Zaykin 1999)], and 2) to assign parentage of individuals based upon genotypes from 10-12 microsatellite loci. Maximum likelihood (Marshall et al. 1998) and Bayesian (Neff et al. 2001; Lange 1997) procedures will be used to exclude possible crosses and parents (parental exclusion analysis). The software program FaMoz (Gerber et al. 2003) will be used for this analysis.

2.5 Reconditioning

Objective 3. Apply kelt steelhead reconditioning techniques at selected streams to post-spawners for release back into study streams.

2.5.1 Steelhead Kelt Collection

At each study site, kelt steelhead will be collected as they accumulate on the upstream side of each picket weir. These fish will be removed with dip nets and placed in an anesthetic tank. Anesthetized steelhead will be visually examined to classify each fish as a kelt or prespawn individual. Methods for visual classification are available (Hatch et al. 2002) and primarily involve keying specimens based on an imploded abdomen. This visual technique was highly precise when compared with the use of ultrasound analysis (Evans 2002). If a specimen is suspected to be a pre-spawner the fish will be released on the downstream side of the weir. Following collection, fish will be transported to reconditioning facilities and then anaesthetized, to be "in-processed", where they are scanned for a PIT tag, measured, weighed, fish color and condition noted, intubated Ivomec (parasite treatment), and injected with a PIT tag if not present in the specimen. The kelts are then released into 10' circular holding tanks. In closed aquatic environments, such as kelt reconditioning tanks, severe infestation of parasites can be lethal to cultured fishes, which may be especially susceptible to *Salmincola* in such environments. *Salmincola* is a genus of parasitic copepods that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface by attachment to the lamellae. Recent research by Johnson and Heindel (2000), suggested that Ivermectin™ – a treatment often used to control parasites in swine and cattle – can potentially increase the survivorship of cultured fish by killing the adult morph of the parasite. Due to its successful use in treating *Salmonicola* at the Prosser Hatchery Facility reconditioning experiments during 2000 (Evans et al. 2000), Ivermectin™ will be diluted with saline (1:30) and injected into the posterior end of the fish's esophagus using a small (10cc) plastic syringe.

2.5.2 Holding

After spawning naturally post spawn steelhead will be collected as they wash up on enumeration weirs. They will then be transferred to a small truck holding tank that will circulate (during capture) or recirculate (during transport) river water to keep fish cool and well oxygenated. It is anticipated that fish will be held for less than a 12-hour period before being transported to reconditioning facilities.

2.5.3 Transporting

Fish will be transported from the capture areas to recondition facilities daily. We determined appropriate holding time based on keeping stress to minimal levels. We used these criteria to determine if it was necessary for kelt steelhead to be transported in standard fish tankers with injected oxygen or smaller tanks hauled by truck.

2.5.4 Feeding / Reconditioning

From our experience with reconditioning kelt steelhead the preferred container are 20 ft (diameter) large circular tanks (Hatch et al. 2002). Individual tank carrying capacity has been estimated at 200 fish based on the aquaculture experience of YN hatchery staff, and the project goal of maximizing kelt survival in captivity. Formalin will be administered five times weekly at 1:6,000 for 1 hour in all reconditioning tanks to minimize fungal outbreaks. Initially, a diet of frozen krill will be fed to the kelt steelhead followed by a maintenance diet of Moore-Clarke salmon pedigree diet. Experience at Prosser Hatchery has demonstrated that frozen krill was superior to starter paste diets in eliciting feeding behavior (Hatch et al. 2002). Kelts that received krill as a starter diet had an average survival rate of 45% compared to only 28% survival of kelts not exposed to krill in 2001 experiments. Despite the apparent advantages of krill, a maintenance feed was necessary to augment rematuration rates. In the absence of a maintenance diet, re-maturation rate was only 10% compared to a 27% rematuration rate with a maintenance diet. In general, results indicated that frozen krill followed by Moore-Clarke salmon pedigree diet provided the best overall survival and rematuration rates in 2001.

2.5.5 Evaluation / Release

Surviving reconditioned kelt steelhead will be released above weir sites during the following fall migration. Prior to release each kelt will be anesthetized and examined with ultrasound equipment to determine maturation. Ultra-sound image captures of each fish will be stored electronically and later individual egg size will be determined. Such data may be used for comparison between hatchery-origin and natural-origin individual since it has been reported that hatchery-origin fish tend to produce smaller eggs (Heath et al. 2003). Data such as PIT tag number, length, weight, marks, and condition by individual will be recorded.

The following data will be collected on all kelts that die during the reconditioning process at the three reconditioning facilities. On discovery of a mortality, fish will be first subjected to an external examination by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies.

Once the external exam is completed, an internal examination will be conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. Any PIT, acoustic and/or radio tags will be removed from mortalities and identification numbers entered into a computer database along with the growth measurement data. We will attempt to reuse viable tags whenever possible.

Reconditioning rates will be determined by the ratio of the number of fish released alive to the number of kelts initially stocked in the tank. Rematuration rates will be calculated by the number of mature fish at release divided by the total number of fish released. We will use two-way analysis of variance to test for differences in reconditioning and rematuration among study sites.

3.0 RESULTS

3.1 Planning and Coordination

3.1.1 Steelhead Prespawner Collection

After meeting with Tribal Fisheries Staff from the three Tribes involved, site locations for weir placement were determined. Field collections will be made at the following sites: Shitike Creek, a tributary to the Deschutes River (The Confederated Tribes of Warm Springs); Omak Creek, a tributary to the Okanagon River (Confederated Tribes of the Colville Reservation); and a tributary of Satus Creek (Yakama Nation). At each collection site juvenile steelhead and resident *O. mykiss* will be sampled as well as all adult steelhead (pre-spawner and post-spawner). All necessary permitting for weir placement has been accomplished. Yakima and Colville are currently fabricating weirs for placement and should be finished shortly, for weir testing. Testing of weirs for collection efficiency and operation will be finished by late 2004.

3.1.2 Steelhead Kelt Collection

Steelhead kelt collection will take place at the aforementioned sites. The Columbia River Inter-Tribal Fish Commission has meet with each of the Tribal organizations to determine the logistics necessary for the capture steelhead kelts at these locations. After spawning naturally post-spawn steelhead (kelts) will be collected as they wash up on enumeration weirs. They will then be transferred to a small truck holding tank that will use circulated (during capture) or recirculated (during transport) river water to keep fish cool and well oxygenated. It is anticipated that fish will be held for less than a 12-hour period before being transported to reconditioning facilities.

3.1.3 Steelhead Kelt Reconditioning

We investigated two different approaches for reconditioning. The first approach was to see how feasible centralized reconditioning would be. Our logic was based upon the assumption that the expertise of the Yakama crew would be beneficial in reducing cost and achieving a high level of reconditioning success. Our major hurdle to this approach was obtaining the permits necessary for moving out of basin fish to our reconditioning site. After our initial investigation we learned that it would be highly difficult to nearly impossible to obtain the necessary permits for transporting out of basin fish. Potential disease transfer or fish escape from one basin to another were the greatest concerns (especially with out of state fish), which would require an extensive permitting and approval process.

The alternative of reconditioning near each site was determined was the most feasible. Obtaining suitable reconditioning sites was not as difficult as anticipated. The reconditioning of steelhead kelts will be conducted at the following facilities: Cassimer Bar Fish Hatchery for Omak Creek captured fish, Prosser Fish Hatchery for Satus Creek captured fish, and Warm Springs National Fish Hatchery for Shitike Creek captured fish.

3.1.4 Juvenile O. mykiss Collection

Rotary trap locations for Omak and Shitike Creeks were already in place prior to the advent of this project. We will continue to utilize these locations to capture juvenile steelhead. Due to stream size limits of the Satus Creek tributaries in which adults will be released we are not able to use a screw trap so seine nets will be used to collect juveniles. Currently we are in process with NOAA Fisheries obtaining ESA collection permits. The two screw traps in place have been proven to be effective at capturing juvenile steelhead in these basins, as they have captured juvenile steelhead unintentionally before. The efficiency of using a small seine net has been proven effective at capturing juveniles (sampling technique citation).

3.1.5 Resident *O. mykiss* Population Collection

It will only be necessary to capture resident *O. mykiss* from Omak and Shitike Creeks. Steelhead from the Satus Creek area will be released above an immigration barrier in two of the four 4 tributaries of Satus Creek (North and South Fork Logy, Section Corner, and/or Yatamai Creeks) that has no known resident *O. mykiss or Salvelinus confluentus (bull trout)* populations (Pers. Communication Yakima Fisheries Staff, 2004). As steelhead are placed in these tributaries, resident O. mykiss populations may establish but should not become contributing members to the spawning population until 2008-2009.

3.1.6 Genotyping

Test runs will be done in late 2004 to allow us to optimize loci and multiplex sets. Results will be published in the 2005 annual report.

3.1.7 Project Coordination

In order to facilitate research on Tribal lands approval must be obtained from the respective Tribe's Fish and Wildlife Committees. We have received permission to proceed with research from these Tribal organizations. Ongoing coordination and updates on project progress will continue to be presented to these organizations. Along with the Tribal approval process subcontracting with each of the Tribal Fish and Wildlife Departments was necessary to pay for fieldwork and obtaining raw experimental data. All subcontracts are in place for 2004-2005 work. Most equipment has been purchased or is in process of being purchased by late 2004, in preparation for the 2005 field season. All proper testing of equipment has and will be done to ensure proper working function. Protocols to ensure consistent sampling procedures, collections, tagging, and handling are listed in Appendix A. All necessary permit acquisition is currently in process for handling of ESA listed species. This report and the 2005 statement of work will be in place and delivered by late 2004 for the 2004-planning year and 2005 field season.

3.2 Reproductive Success

3.2.1 Parentage Assignment

Initial results from 2005 will be published in 2005 annual report.

3.2.2Generalized Sampling Scheme

Initial results from 2005 will be published in 2005 annual report.

3.2.3 Laboratory Techniques

Any changes in laboratory techniques will be reported in subsequent reports.

3.2.4 Statistical Analysis

Initial Analysis should be available by either late 2006 or 2007 and will be reported in that years corresponding annual report.

3.3 Reconditioning

3.3.1 Steelhead Kelt Collection

Initial results from 2005 will be published in 2005 annual report.

3.3.2 Feeding / Reconditioning

Initial results from 2005 will be published in 2005 annual report.

3.3.3 Evaluation / Release

Initial results from 2005 will be published in 2005 annual report.

4.0 Conclusion

In 2004 our main focus was to coordinate and determine the best reconditioning sites based on our resources. So far our efforts at coordination and approval from various agencies involved has gone well and appears to be well on track to a successful 2005 field season. We also developed an operations plan to facilitate successful coordination at the various reconditioning facilities. To date steelhead kelt reconditioning appears to be a promising tool to bolster and potentially recover struggling steelhead populations and unique life history trait (kelting). These experiments should test the efficacy of this technique to improve steelhead populations and improvement of the kelt life history trait. We look forward to seeing the results of this study and continuing to search for a solution to the population and life history loss that is afflicting steelhead kelts in the Columbia River Basin.

5.0 References

Allendorf, F. W., and 9 co-authors. 1997. Prioritizing Pacific salmon stocks for conservation. Conservation Biology 11(1):140-152.

Bell, G. 1980. The costs of reproduction and their consequences. The American Naturalist 116(1):45-76.

Bernatchez, L. and P. Duchesne. 2000. Individual-based genotype analysis in studies of parentage and population assignment: How many loci, how many alleles? Canadian Journal of Fisheries and Aquatic Sciences 57:1-12.

Chebanov, N. A. and B.E. Riddell. 1998. The spawning behavior, selection of mates, and reproductive success of Chinook salmon (Oncorhynchus tshawytscha) spawners of natural and hatchery origins under conditions of joint spawning. Journal of Ichthyology. 38:517-526.

Chilcote, M.W., Leider, S.A., Loch, John J., 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans. Am. Fish. Soc. 115:726-735.

Eldridge, W. H., M. D. Bacigalupi, I. R. Adelman, L. M. Miller, and A. R. Kapuscinski. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. Canadian Journal of Fisheries and Aquatic Sciences 59:282-290.

Estoup, A, K. Gharbi, M. SanChristobal, C. Chevalet, P. Haffray, and R. Guyomard. 1998. Parentage assignment using microsatellites in turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) hatchery populations. Canadian Journal of Fisheries and Aquatic Sciences 55:715-725.

Eskelinen, P. 1989. Effects of different diets on egg production and egg quality of Atlantic salmon (*Salmo salar* L.). Aquaculture 79: 275-281.

Evans, A.F., and R.E. Beaty. 2000a. (DRAFT) Identification and enumeration of steelhead (Oncorhynchus mykiss) kelts at Little Goose Dam juvenile bypass separator, 1999.

Evans, A.F., and R.E. Beaty. 2000b. An evaluation of steelhead kelt outmigration from Lower Granite Dam to Bonneville Dam to assess downstream conversion rates (survival), routes of passage, and travel times. Proposal for 2001 to US Army Corps Engineers, Walla Walla Dist.

Evans, A.F. and R.E. Beaty. 2001. Identification and enumeration of steelhead (*Oncorhynchus mykiss)* kelts in the juvenile collections systems of Lower Granite and Little Goose dams, 2000. Ann. Rep. To US Army Corps of

Engineers, Walla Walla District, for Contract No. DACW-00-R-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

Evans, A.F. 2002. Steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam: Abundance, downstream conversion rates, routes of passage, and travel times. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-01-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Fleming, I.A. and M.R. Gross (1989) Evolution of adult female life history and morphology in a Pacific salmon (Coho: *Oncorhynchus kisutch*). *Evolution* 43: 141-157.

Fleming, I.A. and M.R. Gross. 1993. Breeding success of hatchery and wild coho salmon (*Oncorhynchus kisutch*) in competition. Ecological Applications 3: 230-245.

Fleming, I.A., A. Lamberg, and B. Jonsson. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. Behav. Ecol. 8(5): 470-480.

Fleming, I.A. 1998. Pattern and variability in the breeding systems of Atlantic salmon (*Salmo Salar*), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55:59-76.

Fleming, I.A., K. Hindar, I. Mjolnerod, B. Jonsson, T. Balstad and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society of London B 267: 1517-1523.

Fleming, Ian, Petersson, Erik, 2001. The ability of released hatchery salmonids to breed and contribute to the natural productivity of wild populations, Nordic Journal of Freshwater Research, 75:71-98.

Gerber S., Chabrier P., Kremer A. (2003) FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers, Molecular Ecology Notes, in press.

Gunsolus, R.T. and G. J. Eicher. 1970. Evaluation of fish-passage facilities at the North Fork project on the Clackamas River in Oregon. Research report to the Fish Commission of Oregon, Oregon Game Commission, United States Bureau of Commercial Fisheries, United States Bureau of Sport Fisheries and Wildlife, and Portland General Electric.

Hatch, D.R., P.J. Anders, A.F. Evans, J. Blodgett, B. Bosch, D. Fast., and T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2001 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Hatch, D.R., R. Branstetter, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2003a. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). Annual Report to the Bonneville Power Administration for Contract No. 00004185. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

Hatch, D.R., R. Branstetter, and S. Narum. 2003b. Evaluate steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam and test the use of transportation to increase returns of repeat spawners. Annual Report to US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-00-C-0027. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

Hambrook, M.J., and G.M. Hare. 1993. New uses for kelt. *In* Salmon in the Sea and New Enhancement Strategies. *Edited by* D. Mills. Oxford: Fishing News Books, Blackwell Scientific Publications. pp. 332-337.

Heath, D. D. John W. Heath, Colleen A. Bryden, Rachel M. Johnson, and Charles W. Fox. Rapid Evolution of Egg Size in Captive Salmon. Science 2003 March 14; 299: 1738-1740.

Hockersmith, E. J. Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetry study: Steelhead, 1989-93. Report to US Dept. Energy, Bonneville Power Administration, for Proj. No. 89-089, Contract No. DE-AI79-89BP00276, by Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA.

ISAB (Independent Science Advisory Board). 2002. Hatchery surpluses in the Pacific Northwest. Fisheries 27(12):16-28.

ISRP (Independent Scientific Review Team). 1999. Scientific issues in the restoration of salmonid fishes in the Columbia River. Fisheries 24(3):10-19.

Johnson, K.A., and J.A. Heindel. 2000. Efficacy of manual removal and ivermectin lavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. Eagle Fish Health Laboratory and Hatchery report, Idaho Department of Fish and Game, Boise.

Lange, K. 1997. *Mathematical and Statistical Methods for Genetic Analysis*. Springer-Verlag, New York, 265p.

Leider, S. A., P. A. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239-252.

Letcher B.H. and T.L. King. 2001. Parentage and grandparentage assignment with known and unknown matings: application to Connecticut River Atlantic salmon restoration. Can. J. Fish. Aquat. Sci. 58(9): 1812-1821.

Lewis, P. O., and D. Zaykin. 1999. GDA: Genetic Data Analysis (version 1.2) free program distributed by the authors at the GDA homepage: http://chee.unm.edu/gda/

Marshall, T. C. J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

Narum, S. R., C. Contor, A. Talbot and M. Powell. *Submitted*. Genetic divergence of sympatric resident and anadromous forms of *Oncorhynchus mykiss* in the Walla Walla River and Columbia River Basin, USA. Journal of Fish Biology.

Neff, B. D., J. Repka, and M. R. Gross. 2001. A Bayesian framework for parentage analysis: The value of genetic and other biological data. Theoretical Population Biology 59:315-331.

Nehlsen, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Washington, and Idaho. Fisheries 16: 4-21.

NMFS (National Marine Fisheries Service). 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. Seattle, WA.

Norris, A. T., D. G. Bradley and E. P. Cunningham. 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture 182:73-83.

NPPC (Northwest Power Planning Council). 1986. Compilation of information on salmon and steelhead losses in the Columbia River Basin. Portland, OR. 252 p.

NPPC (Northwest Power Planning Council). 1995. 1994 Columbia River Fish and Wildlife Program (revised 1995). Portland, Oregon.

NPPC (Northwest Power Planning Council). 1999. Artificial Production Review: Report and recommendations of the Northwest Power Planning Council. Portland, OR. Document 99-15.

National Research Council. 1996. Upstream: Salmon and society in the Pacific Northwest. National Academy Press, Washington D.C.

O'Reilly, P.T., C. Herbinger and J.M. Wright. 1998. Analysis of parentage determination in Atlantic salmon (*Salmo salar*) using microsatellites. Animal Genetics 29:363-370.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population geneticssoftware for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Savvaitova K. A., K. V. Kuzishchin, S. V. Maksimov, and S. D. Pavlov. 1996. Population Structure of Mikizha, Salmo mykiss in the Utkholok River (Western Kamchatka). Journal of Ichthyology : 37(3) 216-225.

TRP (Tribal Restoration Plan). 1995. Wy-Kan-Ush-Mi Wa-Kish-Wit: The Columbia River anadromous fish restoration plan of the Nez Perce, Umatilla, Warm Springs, and Yakama tribes. Columbia River Inter-Tribal Fish Commission, Portland, OR

U.S. v. Oregon. 1997. 1996 all species review, Columbia River fish management plan. Technical Advisory Committee. Portland, OR.

Wertheimer, R.H., P.L. Madson, M.R. Jonas, and J.T. Dalen. 2002. Evaluation of steelhead kelt project abundance, condition, passage, and conversion rates through lower Columbia River dams, 2001. Report of Monitoring US Army Corps of Engineers, Portland District Fisheries Field Unit, Bonneville Lock and Dam, Cascade Locks, OR.

Wingfield, W. 1976. Holding summer steelhead adults over to spawn second year. 27th Northwest Fish Culture Conference. Twin Falls, ID.

Withler I. L. 1966. Variability in life history characteristics of steelhead trout (*Salmo gairdneri)* along the Pacific Coast of North America. Journal of the Fisheries Research Board of Canada 23: 365-393.

Whitt, Charles R. 1954. The Age, Growth, and Migration of Steelhead Trout in the Clearwater River, Idaho. Thesis submitted to the University of Idaho Graduate School.

Appendix

Appendix A.

Operations and Procedures:
An Evaluation of the Reproductive Success of Natural-Origin, Hatchery-Origin, and Kelt Steelhead in the Columbia Basin
Contract No. 16530
Project Number 2003-062-00

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Table of Contents

1.0 Introduction

1.1 Background

Reconditioning is the process of capturing emigrating post-spawned mature adult steelhead (aka. kelt) and holding them either for a short or long-term period and feeding them a formulated diet that has demonstrated effective rematuration and survival rates. Reconditioning of steelhead kelts began in 2000 (Evans *et al.* 2001) with a number of experiments the following years to the present since then we have significantly increased rematuration and survival of these fish. This manual is meant to be a guide to the reconditioning process and provide the most recent techniques used to recondition steelhead kelts. For further information on previous studies please see: http://www.critfc.org/tech/tech_rep.html.

1.2 Purpose

The purpose of this study is to determine if effective reconditioning strategies devised for Yakima River steelhead kelts can be replicated throughout the greater Columbia River basin on different stocks located at different basins to increase iteroparity (reproducing more than once during lifespan) thus bolstering ESA-listed or potential candidate populations.

2.0 Objectives and Tasks

2.1 Facilities

All equipment used in the process of capturing, transport, and reconditioning of steelhead kelts should in all possible cases adhere to the established Integrated Hatchery Operations Team (IHOT) standards to prevent and reduce the chance of disease transmission to experimental specimens. Any surface that comes into continuous contact with fish should be decontaminated (i.e. nets, boots, tanks) we suggest Argetyne Iotafor ™ or equivalent decontaminant. It is essential that flows are not too high, we suggest based on a 20ft circular tank that flows should be in 100- 300 gallon/min average, with 200 gallons/min being the preferred rate. Tanks should also be lined before kelts are placed into tanks to reduce potential eyed damage. A polyurethane truck bed liner was applied in 2004 utilizing a specific color scheme (the bottom is blue in color with the tank walls white) to reduce the occurrence of eye damage. This method for reducing eye damage so far looks to be promising, results will be published in the 2005 Steelhead Kelt Reconditioning Annual Report.

2.2 Collection and Transport

2.2.1 Collection

All steelhead captured in the field should be handled with extreme care and promptness. Traps should be checked as much as possible for any potential captures. Time is of the essence in captures, this will be especially true for steelhead kelts that are weakened from lack of food and do not have much energy to fight a current when trapped on a picket weir. All fish captured (target and non-target) should be handled with care (use of a dipnet or fish bag) and either promptly released or transferred to a sampling trough (please see genetic samples section below) then/or holding tank. We will only capture fish that appear in fair to good physical condition, due to the poor reconditioning performance of fish found to be in poor condition. Literature and project experience support the suggestion that a positive correlation between handling time and mortality exist.

2.2.2 Genetic Sampling

All specimens selected for sampling will have 5 mm caudal punch taken for genetic sampling to determine or assign parentage of sampled fish. If fish are to small for a caudal punch a small clip of the fin will be used to identify parentage of the offspring. Collected samples will be stored in small 1.5 ml containers that have a 1-1.2 ml of an ethanol or lysis buffer for preservation of DNA. Samples should be shipped as soon as possible to the Hagerman Fish Culture Experiment Station in care of Shaun Narum at Hagerman, ID.

2.2.3 Holding

Fish in holding tanks should be kept cool if ambient temperatures are high with maximum water temperature not to exceed 63^0 F by using either by circulating river water, a chiller, or ice (non-chlorinated). Oxygenation of water may also be necessary, it is suggested that oxygen levels should be maintained at 5 ppm.

2.2.4 Transport

Steelhead kelts should be transported in a safe and timely manner so as to avoid additional stress to fish.

2.3 In-Processing

In processing is when fish are brought to reconditioning facilities to be fed so as to initiate rematuration. After removal from the holding tank, fish are to be moved via dipnet or fish bag to a sampling tank containing fresh river water or well water, and anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 ppm. Following kelt identification, we will collect data on weight (recorded in lbs.), length (fork length recorded in inches), condition (good- lack of any wounds or descaling, fair- lack of any major wounds and/or descaling, poor- major wounds and/or descaling), coloration (bright, medium, dark), and presence or absence of physical anomalies (e.g., head burn, eye damage). Passive Integrated Transponder (PIT) tags (if not already present) will be implanted in the fish's abdominal cavity for individual fish identification to measure success of reconditioning.

So directly after obtaining data on steelhead kelts and just before release into reconditioning tanks an antiparasitic and antibiotics are administered to prevent a possible epidemic. In kelt reconditioning tanks, severe infestation of parasites can be lethal to cultured fishes, steelhead may be especially susceptible to *Salmincola* in such environments. *Salmincola* is a genus of parasitic copepod that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface by attachment to the lamellae. Recent research by Johnson and Heindel (2000), suggested that Ivermectin™ – a treatment often used to control parasites in swine and cattle – increases the survivorship of cultured fish by killing the adult morph of the parasite. Due to its successful use in treating *Salminicola* in this project's kelt reconditioning experiments during 2000 (Evans and Beaty 2000), Ivermectin™ was diluted with saline (1:30) and injected into the posterior end of the fish's esophagus using a small (1cc) plastic syringe. Fish also received a single .25ml treatment of Oxytetracycline.

2.4 Reconditioning

2.4.1 Tank Sanitation

Optimal reconditioning tank temperature is suggested to be maintained at 57^0F with maximum temperatures not exceed 63^0 F; failure to follow temperature guidlines may result in unacceptable mortality rates. Formalin will be administered five times weekly at 1:6,000 for 1 hour in all reconditioning tanks to minimize fungal outbreaks. Tanks should also be cleaned every 10 days. First drop the water level to an acceptable level and then use a crowder to protect fish from any potential injury. Once this is done the tank should be scrubbed to remove grime from the tank walls.

Tanks should be monitored for mortalities with any mortalities being promptly removed to prevent unsanitary conditions. After removal, a quick necropsy should be performed to ascertain cause of mortality, with suspected cause of mortality recorded. The following data will be collected on all kelts that die during the reconditioning process at the three reconditioning facilities. On discovery of a mortality, fish will be first subjected to an external examination by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. Once the external exam is completed, an internal examination will be conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. Any PIT, acoustic and/or radio tags will be removed from mortalities and identification numbers entered into a computer database along with the growth measurement data. We will attempt to reuse viable tags whenever possible. Mortalities should then be properly disposed of in accordance with federal, state, and tribal regulations.

2.4.2 Feeding

Initially, a diet of frozen krill will be fed to the kelt steelhead (2 months) followed by a maintenance diet of Moore-Clarke salmon pedigree diet. Steelhead kelts are fed 4 to 5 times daily at 2 lbs per each feeding for the krill starter feed and then fed 3 times daily at 5 lbs per each feeding for the Moore-Clark pellet maintenance feed. The diet supplement hw-wiegandt multi vit may also be administered in steelhead kelt reconditioning, it has shown promise when used with krill to initiate feeding. To this date it has been administered experimentally for the first 2 weeks of reconditioning.

2.5 Evaluation and Release

Surviving reconditioned kelt steelhead will be released above weir sites during the fall of the year. Prior to release each kelt will be anesthetized and examined with ultrasound equipment to determine maturation. Ultra-sound image captures of each fish will be stored electronically and later individual egg size will be determined. Such data may be used for comparison between hatchery-origin and natural-origin individual since it has been reported that hatchery-origin fish tend to produce smaller eggs (Heath et al. 2003). Data such as PIT tag number, length, weight, marks, and condition by individual will be recorded.

3.0 References

Evans, A.F., R.E. Beaty, D.R. Hatch, J. Blodgett, and D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Heath, D. D. John W. Heath, Colleen A. Bryden, Rachel M. Johnson, and Charles W. Fox. Rapid Evolution of Egg Size in Captive Salmon. Science 2003 March 14; 299: 1738-1740.

Johnson, K.A., and J.A. Heindel. 2000. Efficacy of manual removal and ivermectin lavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. Eagle Fish Health Laboratory and Hatchery report, Idaho Department of Fish and Game, Boise.

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